A Rapid and Sensitive *In Vitro* Bioassay of Follicle Stimulating Hormone: Estradiol-17β Formation by Dispersed Seminiferous Tubule Cells from Immature Rats

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ABSTRACT—The aromatase activity of Sertoli cells enriched cultures from immature rat testis has recently been utilized for *in vitro* bioassay of follicle stimulating hormone (FSH), with conversion of exogenous 19-hydroxyl-androstenedione (19OH-A) to estradiol-17β being used as end point. The process of the preparation of cultured Sertoli cells was, however, rather lengthy. We have simplified the bioassay system, by directly employing the dispersed seminiferous tubule cells, without initial culture of the Sertoli cells for 2–4 days, for incubation with FSHs. Such simplified incubation system was found to be rapid, sensitive and specific for *in vitro* bioassay of purified FSH preparations. In brief, decapsulated testes, obtained from 12 days old rats, were dispersed with collagenase for 10 min in Medium A. The dispersed seminiferous tubule cells were distributed into the multiwell of culture plate, preincubated with 19OH-A for 2 hr and then incubated with varying doses of FSHs in Medium B (Mediums A + 0.125 mM MIX) at 25°C for 20 hr, under continuous aeration in Dubnoff incubator shaken at 50 cycle/min. Dose related estradiol-17β productions were produced by various mammalian FSHs. Such simplified incubation system is highly reliable and useful for *in vitro* bioassay of FSH activity from purified hormonal preparations or for monitoring FSH activity during isolation of the hormones. Cyclic AMP formation and accumulation by the dispersed seminiferous tubule cells were also investigated. The results indicated that the estradiol-17β formation system is more sensitive than the cyclic AMP production system for *in vitro* FSH bioassay.

INTRODUCTION

Gonadotropins are synthesized in and secreted from pituitary glands of diverse mammalian and nonmammalian vertebrate species [1, 2]. In most vertebrate species, two distinct types of pituitary gonadotropins have been identified, LH and FSH. FSH regulates gonadal maturation, ovarian follicles development and testis seminiferous tubule spermatogenesis [3–8] as well as gonadal steroidogenesis [9–13]. Various biological assay systems have been established for quantification of the FSHs prepared from pituitaries or present in circulation [14, 15]. The classical method for *in vivo* FSH bioassay of Steelman and Pohley [16], based on the augmentation with human chorionic gonadotropin (hCG), has been widely used. Because of its low sensitivity, it is not convenient for

measuring samples of small amount, and for routine assays. To improve the sensitivity, several *in vitro* bioassays for FSHs have been developed for purified preparations or pituitary extracts. These include, the measurement of [³H]-thymidine uptake into mouse ovaries [17], [³H]-glucosamine incorporation into proteoglycans by porcine granulosa cells [18], cAMP production by rat seminiferous tubules [19], plasminogen activator production by rat granulosa cells [20], the stimulation of estradiol production in rat Sertoli cells [10, 15, 21] and aromatase activity in rat granulosa cells [14]. Recently, the stimulating effect of FSH on aromatase activity which converting androgen to estrogen by primary cultures of granulosa cells and Sertoli cells have been improved [15, 22]. FSH specifically stimulates the aromatase activity of the primary cultures of both granulosa cells and Sertoli cells prepared from gonads of immature female and male rats, respectively; in these studies, dose related formations of estradiol-17β are produced

from the added testosterone [10, 11, 21, 22]. Although both assay systems have been improved recently, the whole process is still rather lengthy with respect to the preparation of cultured granulosa cells and Sertoli cells. We thus have attempted to further improve such assay systems. We report here a rapid, sensitive, and reliable *in vitro* FSH bioassay, basing on the estradiol formation activity by dispersed seminiferous tubule cells prepared from immature male rats.

MATERIALS AND METHODS

Hormones and chemicals

Ovine FSH (NIADDK-oFSH-17), human FSH (NIDDK-hFSH-B1), ovine LH (NIADDK-oLH-25), ovine TSH (NIADDK-oTSH-12), ovine PRL (NIADDK-oPRL-18) and ovine GH (NIADDK-oGH-14) were a generous gift of the National Hormone and Pituitary Program, NIADDK, Baltimore, MD. Porcine FSH (USDA-pFSH-B1) was from the USDA Animal Hormone Program, USA. Human menopausal gonadotropin (HMG), pregnant mare serum gonadotropin (PMSG), insulin (from bovine pancreas, for cell culture, 24.5 IU/mg), 1-methyl-3-isobutyl-xanthine (MIX), bovine serum albumin (BSA, Fraction V), HEPES (N-2-hydroxy-ethyl piperazine N-2-ethane sulfonic acid), collagenase (Clostridiopeptidase A; EC 3.4.24.3; type I), penicillin/streptomycin and 19-hydroxyl-androstenedione (19OH-A) were purchased from Sigma Chemical Co. ST. Louis, MO. Medium 199 (with Hanks' Salts and L-Glutamine) was from GIBCO Laboratories, Chagrin Falls, Ohio. Tritiated estradiol-17β (2, 4, 6, 7, 16, 17-[^{3}H], 55 Ci/m mol) and cyclic AMP (2, 8-[^{3}H]; 45 Ci/m mol) were purchased from the New England Nuclear Co. Boston, MA. The specifications and biological potencies of the hormones assayed

TABLE 1.   Source, specifications, sensitivity and relative potencies of the hormones assayed in the present FSH bioassay

Hormones	Source	Specified potencies	Sensitivity[a] (ng)	Relative[b] Potency	(95% Fiducial[b] limit)	Linear range[a] of doses (ng)
Human FSH (NIDDK-hFSH-B-1)	NIH	1,683 IU/mg (×2nd IRP-HMG)	0.1	910	(800–1100)	0.1, 0.2, 0.4, 1, 2
Ovine FSH (NIADDK-oFSH-17)	NIH	20×NIH-FSH-S1 0.05×NIH-LH-S1	1.5	100	(91–112)	1, 2, 4, 10, 20
Porcine FSH (USDA-pFSH-B1)	USDA	2.0×NIH-FSH-B1	14	12	(8–15)	10, 20, 40, 100
PMSG (Sigma G-4877)	Sigma	2620 IU/mg	5.2	30	(15–30)	4, 10, 20, 40
HMG (Sigma G-8760)	Sigma	FSH 47.4 IU/mg LH 52.7 IU/mg	20	5	(3–6)	40, 100, 200, 500
Bovine TSH (NIADDK-bTSH-11)	NIH	TSH 27 IU/mg (FSH<1%)	80	1	(0.7–1.4)	100, 250, 500, 1000
Ovine LH (NIADDK-oLH-25)	NIH	2.3×NIH-LH-S1 (FSH<0.5%)	580	0.2	(0.15–0.29)	500, 1000, 2000
Ovine GH (NIADDK-oGH-14)	NIH	1.9 IU/mg (FSH<2%)	2,000	0.1	(0.086–0.12)	2000, 5000, 10000
Ovine PRL (NIADDK-oPRL-18)	NIH	3.0 IU/mg (FSH<0.5%)	>10,000	<0.001[c]	—	—

[a] The mean base line estradiol-17β production from dispersed seminiferous tubule cells without hormones (controls) plus two standard deviations was the method used to extrapolate the sensitivity.

[b] The relative potency of hormone preparations was estimated by the weighting method suggested by Finney [30] for parrallel line assays, using the ovine FSH (NIADDK-oFSH-17) as a refernce (=100). The data are presented as weighted mean with 95% fiducial limits.

[c] Doses of ovine PRL, up 10,000 ng, did not evoke estradiol-17β production.

[d] Within these doses range, the amounts of estradiol-17β production after logarithmic transformation were plotted against the log doses of hormones, giving a linear dose response curve.

in the present study are summarized in Table 1.

Animals

Immature male Wistar rats (12, 16, 18, 20 and 28 days old) were purchased from the Laboratory Animal Center, National Taiwan University, Taipei, Taiwan. They were sacrificed immediately after delivery to the laboratory. The rats were raised at temperature controlled room ($23 \pm 2°C$) and lighting schedule was 12L:12D. Adult rats were fed with Puina Chows and water supplied *ad libitum*.

Preparation of dispersed seminiferous tubule cells

The method used for the preparation of the cells was modified from the procedures employed by Dorrington *et al.* [23] and Van Damme *et al.* [21]. Briefly, decapsulated testes, from 10 rats of 12 days old were incubated in Medium A (Medium 199 with 25 mM HEPES, penicillin 10,000 units/100 ml, streptomycin 5 mg/100 ml, 0.1% BSA, 10% sodium bicarbonate 1 ml/100 ml, pH 7.40) containing collagenase (2 mg/1 g testis/4 ml) in an incubator shaken at 100 cycles/min at 35°C for 10 min. Seminiferous tubules were settled to the bottom of centrifuge tube (50 ml, Falcon) following addition of another 10 ml of Medium A, and the supernatant was decanted to remove interstitial cells. The seminiferous tubules were repeatedly washed with 10 ml of Medium A for three times, and were then dispersed by collagenase (2 mg/1 g testis/4 ml) again at 35°C for 30 min in a capped Falcon centrifuge tube, placed horizontally, in the incubator shaken at 100 cycles/min. The resulting cell suspension was filtered through 2×2 mm Nylon mesh. The cells were collected by low speed centrifugation ($600 \times g$), and were suspended in 35 ml of Medium B (Medium A with 0.125 mM MIX, pH 7.40). The number of cells was counted with hemocytometer, and viability of the cells was determined by checking its exclusion ability of trypan blue. Viability was routinely greater than 95%. Three hundred microliter of cell suspension containing 2×10^5 viable cells was transferred to each incubation well (16 mm×24 Nonclon).

Sertoli cell aromatase bioassay

Dispersed seminiferous tubule cells were preincubated in Medium B (Medium A with 0.125 mM MIX, pH 7.40) with 150 ng 19OH-A in 100 μl (400 μl/well). After 2 hr preincubation, FSHs or other hormones which were dissolved in 100 μl of Medium B, were added to respective culture well. The total incubation volume was 500 μl per well. Cells were incubated in a Dubnoff incubator shaken at 50 cycles/min with water saturated atmosphere that consisted of 95% O_2 and 5% CO_2 at 35°C for 20 hr. The medium was stored at $-20°C$ freezer until estradiol-17β radioimmunoassay.

Age difference of the Sertoli cell aromatase activity

Immature Wistar rat testes from various age groups (12, 16, 18, 20 and 28 days old) were used as source to investigate the age difference of aromatase activity. Dispersed seminiferous tubule cells were prepared as described previously. Each incubation well contained 2×10^5 cells (in 300 μl), 150 ng of 19OH-A (in 100 μl) and varying doses of oFSH (in 100 μl). The protein contents of cells of each well were extracted with and precipitated by 0.2 N perchloric acid, and were then determined by Lowry method [24].

Time course pattern of estradiol-17β formation

The time course patterns of estradiol-17β formation by dispersed seminiferous tubule cells from 12 days old rats were investigated. Dispersed cells prepared as described previously, incubated with or without 20 ng ovine FSH at 35°C, separately, for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 hr. At the end point of incubation, the incubated media were stored at $-20°C$ until assay.

Radioimmunoassay of estradiol-17β

Preliminary experiments on the quantification of estradiol-17β present in the incubation medium revealed that the estradiol contents were similar either with or without ether extraction. Consequently, the estradiol radioimmunoassay of incubation medium was performed directly without ether extraction. The radioimmunoassay of estradiol-17β was as described previously [25]. The antiserum was produced in rabbits by immunization with estradiol-6-CMO:BSA (Steraloids Inc.). The antibody was highly specific for estradiol-17β.

The cross reactions relative to estradiol-17β (100%) were: estrone, 2.9%; estriol, 0.6%; estradiol-17α, 0.2%; testosterone, 19OH-A, and other steroids had negligible cross reactions with this antiserum [25].

Formation and accumulation of cyclic AMP of dispersed seminiferous tubule cells

A separate study was conducted to examine time course pattern of and dose response in the formation and accumulation of cyclic AMP by the dispersed seminiferous tubule cells upon FSH stimulations. The preparation, preincubation and incubation conditions of the dispersed cells from 12 days old rats were as described previously, but without addition of exogenous 19OH-A. For the time course studies, the cells were incubated with 200 ng of oFSH for various time intervals during the 20 hr incubation period. For the dose response studies, the cells were incubated with varying doses of ovine FSH or ovine LH for 2 hr. The incubated cells and media were then pipetted to glass tubes (12×75 mm) and heated in boiling water for 10 min. The amount of cyclic AMP was determined by competitive protein-binding method [26, 27]. The cyclic AMP binding protein was prepared from fresh bovine adrenal cortex by our laboratory [28] based on Gilman preparation method [29].

Statistical analysis

The statistical calculations of regression, linearity, parallelism and potency estimates between different preparations of the hormones were performed by computer program (STATGRAPHICS) according to the statistical equations and the methods described by Finney [30].

RESULTS

The Effect of methyl-isobutyl-xanthine (MIX) on estradiol-17β formation

The effect of MIX on aromatase activity of dispersed seminiferous tubule cells from 12-day-old rats was investigated. As indicated in Figure 1, dose related estradiol-17β formations were produced by the dispersed seminiferous tubule cells
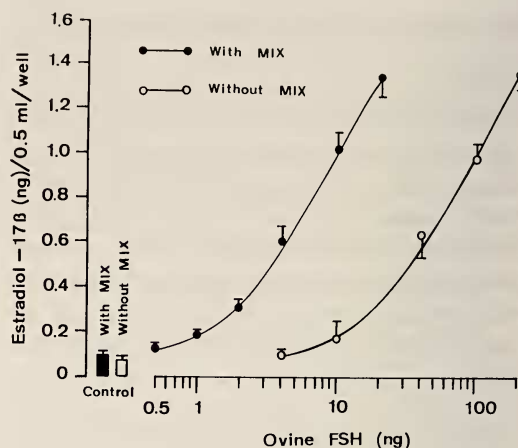

FIG. 1. The effects of 0.125 mM methyl-isobutyl-xanthine (MIX) on estradiol-17β formation of dispersed seminiferous tubule cells prepared from 12 days old rat upon stimulation with varying doses of ovine FSH. The data are expressed as mean ± SEM of triplicate assays from a single incubation experiment.

following 20 hr of incubation with increasing doses of ovine FSH in the presence or absence of 0.125 mM MIX. Addition of 0.125 mM MIX augmented FSH-stimulated estradiol-17β production considerably, and also increased the sensitivity greatly. As a consequence, MIX was included in the incubation system for all experiments in the present study.

Time course patterns of estradiol-17β production

As shown in Figure 2, estradiol-17β production was detectable 2 hr after incubation with 20 ng oFSH, and estradiol-17β was increased more than 10-fold after 20 hr incubation. As indicated, estradiol-17β formation was increasing linearly during the first 12 hr of incubation, and gradually approached the plateau thereafter. The amounts of estradiol-17β production at 20 hr of incubation were approximately 30% greater than those at 12 hr of incubation.

Comparisons of age difference in estradiol-17β formation

The ability and capacity of estradiol-17β formation by dispersed seminiferous tubule cells were compared for rats from 12 through 28 days old at various age groups. As indicated in Table 2, testis
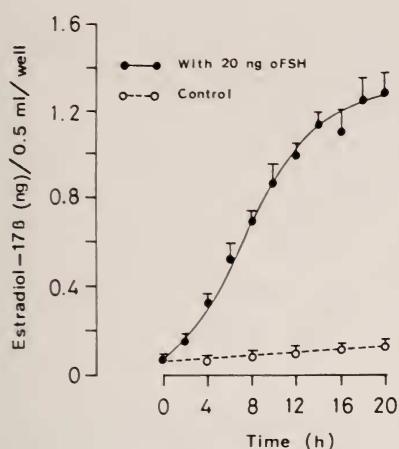
FIG. 2. Time course patterns of estradiol-17β formation from exogenous 19-hydroxyl-androstenedione (19OH-A) by dispersed seminiferous tubule cells of 12 days old rat. The cells (2×10^5 cells/well) were incubated with or without 20 ng of ovine FSH. The data are expressed as mean$\pm$SEM of duplicate assays from three separate incubations.
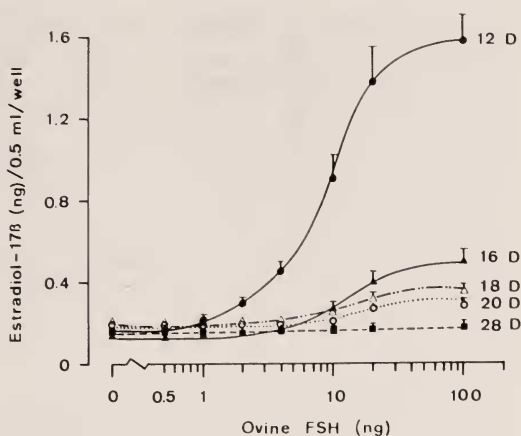

FIG. 3. Age difference of dose related estradiol-17β formation curves of dispersed seminiferous tubule cells (2×10^5 cells/well) prepared from rats at various age groups (12, 16, 18, 20 and 28 days (D) old), in response to ovine FSH stimulation. The data are expressed as mean$\pm$SEM of duplicate assays from three separate incubations.

weight, total cell number and protein content of dispersed cells were all increased with age. In our present study, the Sertoli cells were not identified; thus the number of dispersed cells represented the total number of both Sertoli cells and germ cells. Considerable age difference in estradiol-17β productions was observed. As indicated in Figure 3, the aromatase activity (estradiol-17β formation) expressed on basis of cell numbers per incubation well was decreased with increasing age; similar age pattern of aromatase activity was also observed when expressed on basis of protein content per

incubation well (Data are not shown). The highest aromatase activity was observed in 12 days old rats. Cells from 16, 18, and 20 days old had much lower aromatase activity. The estradiol-17β formation by the cells from 28 days old rats was undetectable. Thus, 12 days old rats were selected in the present study, for provision of Sertoli cells for *in vitro* FSH bioassay.

Hormonal specificity and responses to FSHs and FSH-like gonadotropins from different species

As shown in Figure 4, parallel dose-related

TABLE 2. Comparisons of the testis weight, and cell number and protein content of the seminiferous tubule cells in the immature rat of various age groups[a]

Age (days)	12	16	18	20	28
Body weight (g)	24.3 ± 0.7	28.5 ± 2.6	30.1 ± 1.7	34.5 ± 2.0	56.1 ± 3.4
Testes weight (mg/pair testes)	44.3 ± 3.2	70.5 ± 3.5	97.6 ± 4.5	191.3 ± 8.6	550 ± 9.5
Available cells[b] (10^5/pair testes)	28.2 ± 1.2	36.1 ± 2.5	44.5 ± 3.4	73.0 ± 4.5	280 ± 5.2
Total protein[c]	0.98 ± 0.08	1.26 ± 0.10	2.20 ± 1.16	3.65 ± 0.31	14.0 ± 1.1

[a] The data are expressed as the mean$\pm$SEM from three separate assays.   In each assay, 10 rats of each age group were sacrificed, and the body weight and testes were pooled for each group.
[b] The total number of viable cells prepared from dispersed seminiferous tubules was counted with hemocytometer as described in *Materials and Methods*.
[c] The total protein content (mg/dispersed seminiferous tubule cells of the pair testes) was determined by Lowry method, as described in *Materials and Methods*.
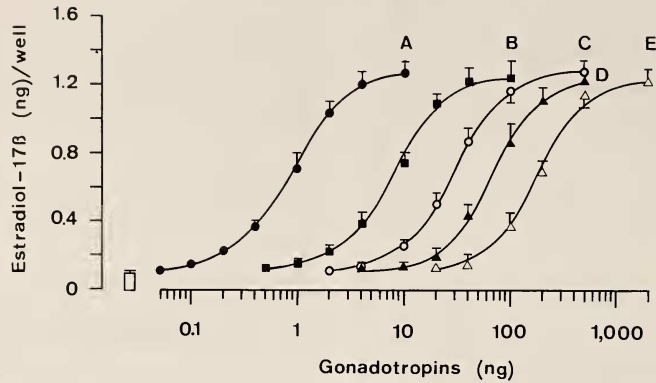
FIG. 4. Comparative potencies of FSHs and FSH-like gonadotropins in estradiol-17β formations by dispersed seminiferous tubule cells prepared from 12 days old rats. A: Human FSH; B: Ovine FSH; C: PMSG; D: Porcine FSH; E: HMG. The specifications of the hormones are indicated in Table 1. The data are expressed as mean±SEM of duplicate assays from three separate incubations.

estradiol-17β formations were produced by human FSH, ovine FSH, porcine FSH, HMG, and PMSG. The potencies of these purified FSHs or FSH-like gonadotropins were different, with human FSH being the most potent. The amounts of estradiol-17β production after logarithmic transformation were plotted against the log doses of hromones, giving linear dose response curves with mean slope 0.780 (0.753–0.827). None of the five dose-response curves significantly nonparallel to each other (p<0.05). In contrast, excessively large amounts of ovine LH, ovine GH and bovine TSH were needed to evoke dose-related estradiol formations (Figure 5). The stimulatory potencies on Sertoli cells aromatase activity of ovine LH, ovine GH and bovine TSH were 0.2%, 0.1% and 1.0% of ovine FSH (NIADDK-oFSH-17), respectively (Table 1). Treatment with ovine PRL (up to 10,000 ng), however, did not stimulate estradiol production by this bioassay system. The precision of this *in vitro* FSH bioassay as characterized by the mean index of precision (λ) was 0.064 in 8 multiple assays of ovine FSH at 3 dose levels. Sensitivity and relative potencies of the tested hormones in the present study are summarized in Table 1.

Cyclic AMP formation and accumulation of dispersed seminiferous tubule cells

From the time course pattern experiment (Figure 6), upon stimulation with 200 ng of ovine FSH, cyclic AMP content was increased during the first 2 hr of incubation, gradually decreased afterwards, and declined to the basal control level at 20 hr of incubation. As shown in Figure 7, dose-related cyclic AMP accumulation was produced by ovine FSH; the maximal response of cyclic AMP production was about 11-fold from the cell controls, without hormonal stimulation, and the lowest detection sensitivity was about 10 ng/culture well, with respect to ovine FSH (NIADDK-oFSH-17). Ovine LH, up to 1000 ng, did not evoke the cyclic AMP formation and accumulation by the dispersed seminiferous tubule cells.
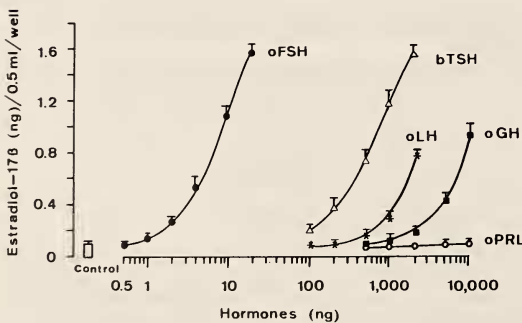

FIG. 5. Hormonal specificity in stimulation of estradiol-17β formation by dispersed seminiferous tubule cells prepared from 12 days old rats. The data are expressed as mean±SEM of duplicate assays from three separate incubations.
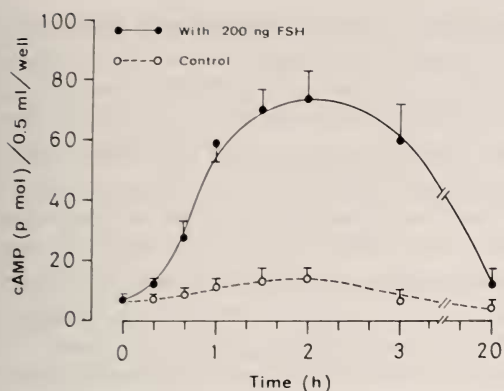
FIG. 6.   Time course patterns of cyclic AMP accumulation of dispersed seminiferous tubule cells of 12 days old rat.   The cells were incubated with or without 200 ng of ovine FSH.   The data are expressed as mean ± SEM of triplicate assays from a single incubation experiment.
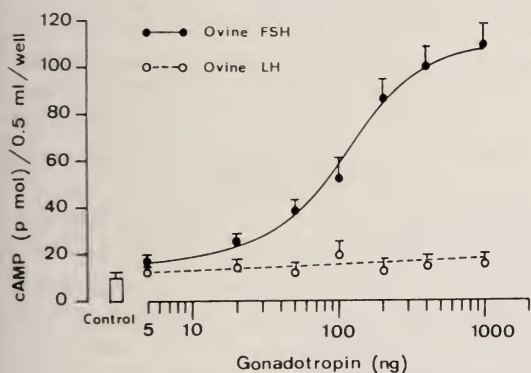

FIG. 7.   Dose response curve of cyclic AMP accumulation of the dispersed seminiferous tubule cells stimulated by ovine FSH.   The cells were incubated in Medium A containing 0.125 mM MIX for 2 hr, at 35°C.   The data are expressed as mean ± SEM of duplicate assays from three separate incubations.

DISCUSSION

The Steelman-Pohley method [16] is the most frequently used assay for measuring FSH bioactivity in pituitary and urinary preparations.   However, the sensitivity of such *in vivo* FSH bioassay is relatively low.   Several *in vitro* FSH bioassay methods have been established [17–22, 31].   Methods have been reported for isolation and maintenance on primary cultures of Sertoli cells

obtained from immature rat testis [10, 11, 23].   These culture cells have been shown to respond to added FSH and dibutyryl cAMP [6] and led to synthesis of estradiol-17β, when testosterone was added to the culture cells [10, 11, 23].   Van Damme *et al.* [21] further applied this *in vitro* system for measurement of biological activity of FSH preparations and of pituitary extracts.   Padmanabhan *et al.* [15] improved this Sertoli cell aromatase bioassay for estimating FSH activity in unextracted human serum.   Jia and Hsueh [22] reported a granulosa cell aromatase bioassay based on FSH-stimulated estrogen production by cultured granulosa cells.   The processes of culture for obtaining both enriched Sertoli cells and granulosa cells were rather lengthy [10, 11, 14]; we have thus attempted to simplify the bioassay system by directly utilizing the dispersed seminiferous tubule cells for incubations, without prior initial culture of cells.

The results from the present study have clearly indicated that the dispersed seminiferous tubule cells *per se,* without initial culture of Sertoli cells, are capable of converting exogenous androgen to estrogen, in a dose-related manner, in response to FSH stimulation.   The estradiol production from cell control to maximal response was about 10-fold.   Our studies also demonstrated that the dispersed seminiferous tubule cells responded, specifically, to FSH, but did not respond to other related hormones such as TSH, LH, PRL and GH, except at extremely excessive amounts.   The hormonal specificity of this FSH bioassay is consistent with the results reported by previous researchers who have employed the cultured Sertoli cells for the bioassay system [15, 21, 32].   Our results also indicated that the responsiveness of dispersed seminiferous tubule cells (Sertoli cells plus germ cells), employed in present study, is similar to that of enriched Sertoli cells used by others [15, 21, 32].   The ability of other cell types, to metabolize testosterone, in the seminiferous tubules from immature rats was previously investigated [6, 23].   They reported that cell suspensions consisting of 85–90% spermatocytes prepared from immature rats formed dihydrotestosterone as a major metabolite of testosterone.   A small amount of 5α-androstane-3α, 17β-diol was also formed, but this

was probably due to the low level of contamination with Sertoli cells. Spermatids, on the other hand, have little, if any, 5α-reductase or 3α-hydroxysteroid dehydrogenase activity. Peritubular myoid cells did not synthesize detectable amounts of estrogen when incubated in the presence of testosterone. These studies provided a basis for us to develop the bioassay system in the present study by directly employing the dispersed seminiferous tubule cells, without initial culture of Sertoli cells.

Signal transduction of gonadotropins is mediated by binding to the membrane receptor and activating cyclic AMP formation [33, 34]. Xanthine, a phosphodiesterase inhibitor, is able to enhance the sensitivity of testis interstitial cell steroidogenesis stimulated by LH [35–37] and granulosa cell estrogen production challenged by FSH [22]. In this study, addition of 0.125 mM MIX greatly enhanced the sensitivity of estrogen production; such results are comparable to those reported by others using different bioassay systems [22, 32].

The sensitivity of the present simplified system is 0.17 mIU of FSH (Figure 4). Such simplified bioassay system is 3- to 6-fold less responsive as compared to the relevant bioassay systems employing Sertoli cells [15, 21, 32] and granulosa cells [22]. In their procedures, the steps of initial culture of the cells for one day [21] or several days [15, 22, 32] were needed prior to stimulation with FSHs. By contrast, in our simplified procedure the initial step of the culture of Sertoli cells was omitted. It has been shown that the sensitivity of the response is greatly increased with increase of the time length of initial culture of Sertoli cells from 24 hr to 124 hr before stimulation with FSHs [15, 21, 22, 32]. Furthermore, it has been demonstrated that the sensitivity of the assay is increased by the addition of insulin, transferrin, epidermal growth factor, hydrocortisone, human chorionic gonadotropin and/or retinoic acid to the culture medium [15]. In our simplified procedure, these factors, however, were not included in the incubation medium. Consequently, these different incubation conditions, as described above, are believed to be the main causes responsible for the different responsiveness between the previous systems and our simplified procedure. By omitting the step of initial culture of Sertoli cells, our simplified bioassay system has thus considerably shorten the length of bioassay from 4–5 working days [31] to 2 working days (this study) without curtailing the sensitivity of the assay to a great extent. Such simplified and rapid incubation system is thus useful for monitoring FSH activities during isolation and characterization of the hormone, and for routine assays of biological activity of purified mammalian FSH preparations. In addition, such system is also convenient and useful for comparisons of FSH activities of pituitary extracts or of purified FSH preparations from various vertebrate classes (will be published elsewhere).

The present results revealed that the effect of FSH on estradiol-17β synthesis by dispersed seminiferous tubule cells prepared from rats of 12 days of age, however, was more dramatic than at 16, 18 and 20 days of age. Dispersed seminiferous tubule cells from rats of 28 days of age synthesized only small amounts of estradiol-17β in the presence of 19OH-A. Such age differences in estradiol-17β formation activity were discernible, regardless expressed either by per unit of protein, or by per unit of total number of cells per incubation well. Our results are generally comparable to the previous findings by Dorrington and Armstrong [6, 10] using the cultured Sertoli cells for incubations. Our results together with previous findings thus indicate that the estradiol formation capacities (or the aromatase activities) of Sertoli cells from rats of 10 to 12 days of age are virtually the same regardless with or without presence of germ cells under the *in vitro* conditions.

Since FSH atimulates adenyl cyclase activity leading to cyclic AMP formation, the determination of cyclic AMP produced by immature rat seminiferous tubule cells has also been employed for FSH bioassay [19, 28, 38, 39]. The results from the present study revealed that cyclic AMP formation was five-fold less sensitive than that of estradiol-17β formation. Estradiol-17β is the end and stable product of aromatase stimulated by FSH in the Sertoli cells; while cyclic AMP, the secondary messenger of the hormone, is rapidly and continuously metabolized within the cells. Conse-

quently, the estradiol-17β formation system is considered to be more reproducible and reliable than the cyclic AMP formation system.

In summary, we have established a simplified, rapid and sentitive *in vitro* FSH bioassay system, measuring estradiol-17β conversion from exogenous 19OH-A by dispersed seminiferous tubule cells prepared from immature rats, without initial culture of Sertoli cells. Such incubation system is suitable for estimating FSH activity from purified hormonal preparations, or for monitoring FSH activity during isolation and characterization of the hormone from pituitary gland or placenta.

REFERENCES

1 Licht, P., Papkoff, H., Farmer, S. W., Muller, C. H., Tsui, H. W. and Crews, D. (1977) Evolution of Gonadotropin structure and function. Recent Prog. Horm. Res., **33**: 169–248.

2 Ryan, R. J., Keutmann, H. T., Charlesworth, M. C., McCormick, K. J., Milius, R. P., Calvo, F. O. and Vutyavanich, T. (1987) Structure-Function relationships of gonadotropins. Recent Prog. Horm. Res., **43**: 383–429.

3 Steinberger, E. (1971) Hormonal control of mammalian spermatogenesis. Physiol. Rev., **51**: 1–22.

4 Greenwald, G. S. (1974) Role of follicle-stimulating hormone and luteinizing hormone in follicle development and ovulation. In "*Handbook of Physiology*" (Eds by E. Knobil and W. H. Sawyer), American Physiology Society, Washington, D. C. Sect. 7, Vol. IV, part 2, pp. 293–323.

5 Ishii, S. and Yamamoto, K. (1976) Demonstration of follicle stimulating hormone (FSH) activity in hypophyseal extracts of various vertebrates by the response of the Sertoli cells of the chick. Gen. Comp. Endocrinol., **29**: 506–510.

6 Dorrington, J. H. and Armstrong, D. T. (1979) Effects of FSH on gonadal functions. Recent Prog. Horm. Res., **35**: 301–342.

7 Richards, J. S. (1980) Maturation of ovarian follicles: Actions and interactions of pituitary and ovarian hormones on folloicular cell differentiation. Physiol. Rev., **60**: 51–58.

8 Hsueh, A. J. W., Bicsak, T. A., Jia, X. C., Dahl, K. D., Fauser, B. C. J. M., Brenda Galway, A., Czekala, N., Pavlou, S. N., Papkoff, H., Keene, J. and Boime, I. (1989) Granulosa cells as hormone targets: The role of biologically active follicle-stimulating hormone in reproduction. Recent Prog. Horm. Res., **45**: 209–277.

9 Ryan, R. J., Petro, Z. and Kaiser, J. (1968) Steroid formation by isolated and recombinated ovarian granulosa and thecal cells. J. Clin. Endocrinol. Metab., **28**: 355–358.

10 Dorrington, J. H. and Armstrong, D. T. (1975) Follicle-stimulating hormone stimulates estradiol-17β synthesis in cultured Sertoli cells. Proc. Natl. Acad. Sci. USA., **72**: 2677–2681.

11 Welsh, M. J. and Wiebe, J. P. (1976) Sertoli cells from immature rats: *In vitro* stimulation of steroid metabolism by FSH. Biochem. Biophys. Res. Commun., **69**: 936–941.

12 Tsang, B. K., Armstrong, D. T. and Witfield, J. F. (1980) Steroid biosynthesis by isolated human ovarian follicular cells *in vitro*. J. Clin. Endocrinol. Metab., **51**: 1407–1411.

13 Hsueh, A. J. W., Adashi, E. Y., Jones, P. B. C. and Welsh, T. H. Jr. (1984) Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocr. Rev., **5**: 76–127.

14 Jia, X. C. and Hsueh, A. J. W. (1985) Sensitive *in vitro* bioassay for the measurement of serum follicle-stimulating hormone. Neuroendocrinology, **41**: 445–448.

15 Padmanabhan V., Chappel, S. C. and Beitins, I. Z. (1987) An improved *in vitro* bioassay for follicle stimulating hormone (FSH): Suitable for measurement of FSH in unextracted human serum. endocrinology, **121**: 1089–1098.

16 Steelman, S. L. and Pohley, F. M. (1953) Assay of follicle-stimulating hormone based on the augmentation with human chorionic gonadotropin. Endocrinology, **53**: 604–611.

17 Boggis, J. and Ryle, M. (1972) An *in-vitro* procedure for the quantitative measurement of follicle stimulating hormone activity. J. Endocrinol., **54**: 355–362.

18 Ax, R. L. and Ryan, R. J. (1979) FSH stimulation of 3H-glucoasamine-incorporation into proteoglycans by porcine granulosa cells *in vitro*. J. Clin. Endocrinol. Metab., **49**: 646–648.

19 Rao, A. J. and Ramachandran, J. (1975) Cyclic AMP production in isolated rat seminiferous tubule cell preparations: A potential *in vitro* assay for follicle stimulating hormone. Life Sci., **17**: 411–416.

20 Beers, W. H. and Strickland, S. (1978) A cell culture assay for follicle stimulating hormone. J.

Biol. Chem., **253**: 3877–3881.

21 Van Damme, M. P., Roberston, D. M., Marana, R., Ritzen, E. M. and Diczfalusy, E. (1979) A sensitive and specific in vitro bioassay method for the measurement of follicle-stimulating hormone activity. Acta Endocrinol. (Copenh), **91**: 224–237.

22 Jia, X. C. and Hsueh, A. J. W. (1986) Granulosa cell aromatase bioassay for follicle-stimulating hormone: Validation and application of the method. Endocrinology, **119**: 1570–1577.

23 Dorrington, J. H., Roller, N. F. and Fritz, I. B. (1975) Effects of follicle-stimulating hormone on culture of Sertoli cell preparations. Mol. Cell. Endocrinol., **3**: 57–70.

24 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem., **193**: 265–275.

25 Yu, J. Y. L., Liaw, J. J., Chang, C. L., Lee, S. N. and Chen, M. C. (1988) Seasonal changes in circulating levels of gonadal steroids during an estrous cycle of Holstein-Friesian Cows. Bull. Inst. Zool., Academia Sinica, **27**: 133–143.

26 Brown, B. L., Albano, J. D. M., Ekins, R. P. and Sgherzi, A. M. (1971) A simple and sensitive saturation assay method for the measurement of adenosine 3′:5′-cyclic monophosphate. Biochem. J., **121**: 561–562.

27 Tsang, C. P. W., Lehotay, D. C. and Murphy, B. E. P. (1972) Competitive binding assay for adenosine 3′,5′-monophosphate employing a bovine adrenal protein: Application to urine, plasma and tissue. J. Clin. Endocrinol. Metab., **35**: 809–817.

28 Yu, J. Y. L. and Song, L. C. (1985) Comparisons of cyclic AMP formation activity of hypophysial follicle stimulating hormones and placental gonadotropins by rat seminiferous tubules in vitro. Proc. 3rd. Cong. ASEAN Fed. Endocr. Soc., Kuala Lumpur, Malaysia, Dec. 1–6, 1985. (Abstract)

29 Gillman, A. G. (1970) A protein binding assay for adenosine 3′:5′-cyclic monophosphate. Proc. Nat. Acad. Sci. USA., **67**: 305–312.

30 Finney, D. J. (1964) Statistical Method in Biological Assay. Hafnet Publishing Co., New York, pp. 58–138.

31 Wang, C. (1988) Bioassays of follicle stimulating hormone. Endocr. Rev., **9**: 374–377.

32 Ritzen, E. M., Froysa, B., Gustafsson, B., Westerholm, G. and Diczfalusy, E. (1982) Improved in vitro bioassay of follitropin. Hormone Res., **16**: 42–48.

33 Roose, E. M. and Gilman, A. G. (1980) Biochemical properties of hormone-sentivitive adenylate cyclase. Ann. Rev. Biochem., **49**: 533–564.

34 Conti, M., Toscano, M. V., Geremia, R. and Stefanini, M. (1983) Follicle-stimulating hormone regulates in vivo testicular phosphodiesterase. Mol. Cell. Endocrinol., **29**: 79–89.

35 Dufau, M. L., Mendelson, C. R. and Catt, K. J. (1974) A highly sensitive in vitro bioassay for luteinizing hormone and chorionic gonadotropin: testosterone production by dispersed Leydig cells. J. Clin. Endocr. Metab., **39**: 610–613.

36 Yu, J. Y. L., Wang, L. M. and Fei, M. L. (1984) Comparative effects of mammalian gonadotropins on androgen formation in vitro from mouse testis interstitial cells. Bull. Inst. Zool., Academia Sinica, **23**: 81–91.

37 Yu, J. Y. L. and Wang, L. M. (1987) Comparative effects of diverse vertebrate gonadotropins on androgen formation in vitro from testes of roosters and mice. Biol. Reprod., **36**: 816–824.

38 Dorrington, J. H., Fritz, I. B. and Armstrong, D. T. (1978) Control of testicular estrogen synthesis. Biol. Reprod., **18**: 55–64.

39 Steinberger, A., Hintz, M. and Heindel, J. J. (1978) Changes in cyclic AMP responses to FSH in isolated rat Sertoli cells during sexual maturation. Biol. Reprod., **19**: 566–572.